Fr. Art. Art. March and the state of the sta

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 2 is a representation of autoradiographs of Western blotting analyses showing that treatment with 40 nM or 80 nM of a representative, non-limiting (SeQ \Rightarrow) ν_0 : i) antisense oligonucleotide, MG88, results in an inhibition of MeTase protein expression in both A549 and T24 cells.

Figure 3A is a representation of autoradiographs of a series of immunoprecipitations followed by Western blotting analyses of T24 cell lysates showing that p16^{ink4} protein levels (upper panel) increase as DNA MeTase protein levels (lower panel) decrease following treatment of the cells for 3, 5, 8, or 10 days with 40 or 75 nM of a representative, non-limiting antisense oligonucleotide of the (SeQ ED No: I) non-limiting antisense oligonucleotide of the invention, MG88, where HeLa cells served as a positive control for p16^{ink4} protein expression.

Figure 3B is a graphic representation of p16^{ink4} protein levels normalized to (SeQ DD No 11) cell number of T24 cells treated with 40 nM or 75 nM MG88 for 3, 5, 8, and 10 days.

Figure 4 is a representation of an autoradiograph of a Western blotting analysis of T24 cells treated with 40 nM or 75 nM of a representative non-limiting (SeQ ID No!) antisense oligonucleotide of the invention, MG88, using an antibody recognizing all phosphorylated forms of Rb.

Figure 5 is a representation of autoradiographs of a series of Western blotting analyses of T24 cell lysates prepared 3, 5, or 7 days after cessation of a 10 (SeQ IDAD!) day treatment of the cells with lipofectin only, 40 nM of MG88, or 40 nM or 75 nM of control oligonucleotide MG208, demonstrating that between 5-7 days post-treatment, DNA MeTase protein expression is restored and p16^{thk4} protein expression is diminished.

Figure 6 is a graphic representation of the quantitation of DNA MeTase and p16^{ink4} protein levels in T24 cells during ten days of treatment with MG88 and 7 days (i.e., days 11-17) post-treatment periods, demonstrating the inverse

THE ATTENDATION OF THE SECOND CONTROL OF THE

5

10

15

20

25

30

р

10

15

20

25

30

relationship between p16 protein levels and DNA MeTase protein levels in these cells.

Figure 7 is a representation of a series photographs of showing the methylation-specific PCR (MSP) products resolved on 2% agarose gels of the plant of the plant

Figure 8 is a diagrammatic representation of the p16^{ink4} proximal promoter from T24 cells showing the methylation patterns after 0, 3, or 5 days of treatment (SeQ IDAG!) of the cells with MG88 (left) or control oligonucleotide MG208 (right), demonstrating that reduced methylation occurred only with MG88 treatment. Day 3 post-treatment methylation patterns are shown at the bottom of the figure.

Figure 9A is a representation of a photograph of the results of bisulfite sequencing of the p16^{ink4} gene promoter in p16^{ink4}-expressing clone MG88 C4-5 30 days following cessation of a 5 day treatment with 75 nM MG88, demonstrating that all CpG sites evaluated were not methylated in this clone even after 30 days in culture post-MG88 treatment.

Figure 9B is a graphic representation showing a growth curve of T24 cells during treatment (days 0-5) and post-treatment (days 6-18) with lipofectin only (SeQ ID Ne!I) (squares), 75 nM MG88, a non-limiting representative antisense oligonucleotide of the invention (circles), or 75 nM or control oligonucleotide, MG208 (triangles), demonstrating the anti-proliferative effect of MG88.

Figure 9C is a graphic representation showing a growth curve during posttreatment of T24 cell clones following a five day treatment with MG88 (clone 4-5, (Cop D) No. 4) triangles), MG208 (clone 2-4, squares), or lipofectin only (clone 5, diamonds). The inserted representation of two autoradiographs show the p16^{ink4} protein level in each of the clones of days 36 and 49 post-treatment.

Figure 10A is a representation of autoradiographs of Western blotting analyses of p21^{WAF1}, MeTase, and α -actin protein levels in T24 cells treated for 24 hours (left panels) or 48 hours (right panels) with lipofectin only, or 40 nM or 75

nM of MG88 or MG208, demonstrating that p21^{WAF1} protein expression is induced by inhibition of MeTase expression.

Figure 10B is a representation of autoradiographs of Western blotting analyses showing the dose-response of p21^{WAF1} protein levels in T24 cells treated for 24 hours with 20 nM, 40 nM, or 80 nM of MG88 or MG208. Actin protein levels are shown as a control for protein loading.

Figure 11 is a representative of an autoradiograph showing a Northern (SeQ I) M_0 : (SeQ II) M_0 : (SeQ III) M_0 : (SeQ II) M_0 : (SeQ II) M_0 : (Se

Figure 12 is a representation of an autoradiograph of a Western blotting analysis showing the reactivation of p16 expression in T24 cells following treatment for three days with increasing concentrations of a representative, non-limiting DNA MeTase protein effector, 5-aza-dC.

Figure 13 is a representation of an autoradiograph of the Western blot analysis showing the synergistic reactivation of p16 expression in T24 cells following treatment for three days with a representative, nonlimiting, synthetic antisense oligonucleotide (MG88) and/or a representative non-limiting DNA MeTase protein effector (5-aza-dC) according to the invention; the three panels show varying combinations and concentrations of representative antisense oligonucleotides and DNA MeTase protein effectors according to the invention.

Figure 14 is a representation of an autoradiograph of the Western blot analysis showing the synergistic reactivation of p16 expression in T24 cells following treatment for three days with a representative, nonlimiting, synthetic antisense oligonucleotide (MG98) and/or a representative non-limiting DNA MeTase protein effector (5-aza-dC) according to the invention; the three panels show varying combinations and concentrations of representative oligonucleotides and DNA MeTase protein effectors according to the invention.

Figure 15 is a representation of an autoradiograph of the Western blot analysis showing the synergistic reactivation of p16 expression in T24 cells following treatment for three days with a representative, nonlimiting, synthetic oligonucleotide (MG88) and/or a representative non-limiting DNA MeTase protein effector (5-aza-dC) according to the invention at low concentrations; the three

A to the second second

5

10

15

20

25

5

10

15

20

25

panels show varying combinations and concentrations of representative oligonucleotides and DNA MeTase protein effectors according to the invention.

Figure 16 is a graphic representation showing the ability of a representative, nonlimiting, synthetic antisense oligonucleotide (MG98) and of a representative, nonlimiting, DNA MeTase protein effector (5-aza-dC) according to the invention to inhibit T24 human bladder cancer cell growth in a synergistic fashion resulting in an increased inhibitory effect as compared to that observed using either only the antisense oligonucleotides or only the DNA MeTase protein effectors.

Figure 17 is a graphic representation showing the synergistic inhibition of T24 human bladder cancer cell growth after treatment for seven days with lipofectin only (first bar from the left); 1 µM of a representative, nonlimiting, DNA MeTase protein effector, 5-aza-dC (second bar from the left); 40 nM of control synthetic oligonucleotide MG207 (third bar from the left); 40 nM of a representative nonlimiting synthetic MeTase antisense oligonucleotide, MG98 (fourth bar from the left); MG207 plus 5-aza-dC (fifth bar from the left); or MG98 plus 5-aza-dC (sixth bar from the left).

Figure 18 is a graphic representation showing the ability of a representative, nonlimiting, synthetic oligonucleotide (MG98) and of a representative, nonlimiting, DNA MeTase protein effector (5-aza-dC) according to the invention to inhibit A549 human non-small cell lung cancer cell growth in a synergistic fashion resulting in an increased inhibitory effect as compared to that observed using either only the oligonucleotides or only the DNA MeTase protein effectors.

Figure 19 is a graphic representation showing the ability of a representative, (SeQ L) No(L) nonlimiting, synthetic oligonucleotide (MG98) and of a representative, nonlimiting, DNA MeTase protein effector (5-aza-dC) according to the invention to inhibit Colo 205 human colon cancer cell growth (expressed as tumor volume over time) in a synergistic fashion resulting in an increased inhibitory effect as compared to that observed using either only the oligonucleotides or only the DNA MeTase protein effectors.

Figure 20A is a graphic representation showing the inhibition of Colo 205 tumor cell growth in nude mice following treatment of the mice with saline (diamond); 0.5 mg/kg of a representative, nonlimiting MeTase synthetic

(SeQ IDM:1)

oligonucleotide, MG98 (square); 0.1 mg/kg of a representative, nonlimiting, DNA MeTase protein effector, 5-aza-dC (triangle); or a combination of both MG98 plus 5-aza-dC (X).

Figure 20B is a graphic representation showing the inhibition of Colo 205 human colon cancer cell growth (expressed as final tumor volume) by a representative, nonlimiting, synthetic oligonucleotide (MG98) and by a representative, nonlimiting DNA MeTase protein effector (5-aza-dC) according to the invention, in a synergistic fashion, resulting in an statistically increased inhibitory effect (p<0.05) as compared to that observed using either only oligonucleotide, protein effector, or saline.

Figure 21 is a schematic diagram showing a series of FACS histogram analyses of T24 cells treated with a representative, nonlimiting, synthetic oligonucleotide (MG88) and/or a representative non-limiting DNA MeTase protein effector (5-aza-dC) according to the invention, at different schedules. The upper panel of histograms shows cells treated on schedule A, where MG88 is administered before 5-aza-dC. The lower panel of histograms shows cells treated on schedule B, where 5-aza-dC is administered before MG88.

Figure 22 is a representation of an autoradiograph of the Western blotting analysis showing the synergistic inhibition of thymidylate synthase protein expression in T24 cells using the combination of a representative, nonlimiting, (SQD) W: 21) synthetic antisense oligonucleotide (MG2605) and a representative, nonlimiting TS protein effector (5-FU) according to the invention.

Figure 23 is a schematic diagram showing a series of FACS histogram analyses of T24 cells treated with a representative, nonlimiting synthetic thymidylate synthase antisense oligonucleotide and/or a representative non-limiting TS protein effector (5-FU) according to the invention. The top histograms shows cells treated with lipofectin only; the second histogram shows cells treated with 25 nM mismatch control oligonucleotide; the third histogram from the top shows cells treated with 25 nM of the TS antisense oligonucleotide, MG2605; the fourth histogram from the top shows cells treated with 500 nM of 5-FU; the fifth histogram from the top shows cells treated with 5-FU plus mismatch

The second of th

5

15

20

25

oligonucleotide; and the sixth histogram (i.e., the bottom histogram) shows cells treated with 5-FU plus the TS antisense oligonucleotide, MG2605.

Figure 24A is a graphic representation showing the percentages of T24 cells in the G_1 phase (gray bars; section M2 on inserted histograms) S phase (black bars; section M3 on inserted histograms), and G_2/M phase (white bars; section M4 on inserted histogram) following treatment with 25 nM of a representative, (SQTD No: 71) nonlimiting TS antisense oligonucleotide MG2605, 25 nM of control oligonucleotide MG2606, 5 μ M of a representative nonlimiting TS protein effector, 5-FU, or a combination of oligonucleotide plus 5-FU.

Figure 24B is a graphic representation of the number of T24 cells remaining following treatment with following no treatment; or treatment with 25 nM of a (SeQIDM: 71) representative, nonlimiting TS antisense oligonucleotide MG2605; 25 nM of control oligonucleotide MG2606; 5 μ M of a representative nonlimiting TS protein effector, 5-FU; or a combination of MG2605 or MG2606 plus 5-FU.

Figure 25 is a representation of an autoradiograph of the Western blotting analysis showing the synergistic induction of p21^{WAF1} by the combination of a representative, nonlimiting, synthetic HDAC antisense oligonucleotide and a representative, nonlimiting HDAC protein effector (TSA) according to the invention.

20

5

10